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Quantification of fibrosis by collagen proportionate area predicts hepatic decompensation in hepatitis C cirrhosis

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SUMMARY

Background
It is unclear whether the course of cirrhosis and its prognosis are related to the amount of collagen in the liver.

Aim
To determine whether fibrosis, assessed by collagen proportionate area (CPA) in patients with compensated cirrhosis, is associated with the presence of oesophageal varices, and predict disease decompensation during the follow-up period.

Methods
We prospectively evaluated 118 consecutive patients with compensated cirrhosis to correlate fibrosis, assessed by CPA in liver biopsies, with the presence of oesophageal varices (OV) and with the rate of liver decompensation (LD) development during a median follow-up of 72 months.

Results
At baseline 38 (32.2%) patients had OV and during the follow-up (median 72 months, IQR 47–91), 17 patients (14.4%) developed LD. The mean CPA value was different in patients with and without OV (14.8 ± 5.9% vs. 21.6 ± 9.5%, $P < 0.001$). The best CPA cut-off for OV by area under the receiver operating characteristic (AUROC) was ≥14% and with multivariate logistic analysis CPA was the only variable associated with OV (OR: 28.32, 95% CI: 6.30–127.28; $P < 0.001$). By AUROC analysis the best CPA cut-off to predict LD was 18.0%. By Cox regression multivariate analysis CPA ≥18% (HR: 3.99, 95% CI: 1.04–11.45; $P = 0.036$), albumin (HR: 0.12, 95% CI: 0.04–0.43; $P = 0.001$) and presence of OV (HR: 8.15, 95% CI: 2.31–28.78; $P = 0.001$) were independently associated with LD.

Conclusion
Quantification of fibrosis by collagen proportionate area allows identification of patients with compensated HCV cirrhosis with a higher likelihood of clinically relevant portal hypertension and a higher risk of decompensation.

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INTRODUCTION

Compensated cirrhosis has a wide range of disease severity,[1] including patients with early, histologically proven cirrhosis, but no clinical evidence of portal hypertension or significant impairment of liver function, as well as patients with large oesophageal varices and a significant risk of liver decompensation (LD). These patients may belong to the same Child–Pugh class, but have a different short and medium-term prognosis.[2] Indeed, 1-year mortality probability in patients with compensated cirrhosis changes according to the presence of oesophageal varices (OV) ranging from less than 1% per year in patients without OV (Stage 1) to 3.4% per year in patients with OV (Stage 2).[2]

The degree of liver fibrosis is one of the most important diagnostic and prognostic assessments in chronic liver disease. Histological assessment of fibrosis is regarded as the 'gold standard' in this respect. However, the changes described in the histopathological scoring categories are largely architectural, with little reference to the quantity, quality and distribution of fibrosis[3, 4] and, also, current scoring systems only have one 'stage' for cirrhosis and no sub-classification is established.[4, 5] Moreover, routine fibrosis assessment is usually carried out on a trichrome or reticulin stain. These are not specific collagen stains, and no measurement of the actual amount of collagen in the liver is made. Computer assisted digital image analysis (DIA) of picroSirius red stained histological sections is a method to quantify liver collagen,[3, 6] as the quantity of picroSirius red correlates well with morphometrically calculated hepatic fibrosis.[7] DIA uses segmentation of digital images to measure the area of collagen and the area of tissue, producing a 'fibrosis ratio' or *collagen proportionate area* (CPA).[3] The reliability of this technique has been recently confirmed by Huang *et al.*[8] who compared liver CPA determined by DIA using different staining methods in 249 patients with chronic hepatitis C. They concluded that CPA using Sirius red stained biopsies was more accurate for quantifying hepatic collagen compared with trichrome staining.

Furthermore, the study of Pilette *et al.*[9] in 1998, have shown a better correlation for all serum direct markers of fibrosis, with the area of fibrosis obtained by image analysis than with the semi-quantitative score. These results suggested that image analysis seems to be the most accurate method for a quantitative approach to liver fibrosis and for reference for non-invasive methods of liver fibrosis assessment. In keeping with these results, other two studies have recently shown a significant correlation of CPA with Liver Stiffness by Transient Elastography and by PSWE (point shear wave elastography technology) in patients with chronic viral hepatitis.[10, 11]

However, CPA evaluation by DIA technique to date has seldom been used clinically, apart from recent studies conducted in a cohort of HCV-infected liver transplant recipients followed with serial biopsies.[12–15] A significant correlation between CPA and Hepatic Venous Portal Gradient was demonstrated both in mild-moderate and severe stages of fibrosis suggesting that CPA can reflect a range of disease severity based on collagen content in this single categorical stage.[12–14]

Moreover CPA was highly predictive of clinical outcome in patients infected with hepatitis C virus who underwent transplantation, better than Ishak stage or HVPG.[13, 14] Finally, analysing changes of CPA in the serial biopsies of the same setting of patients, Manousou *et al.*[15] showed that CPA fibrosis progression rate is a better predictor of clinical outcome than progression by Ishak stage.

The main aim of this study was to determine whether fibrosis, assessed by CPA in biopsies from patients with compensated cirrhosis, was associated to the presence of clinically significant portal hypertension, i.e. oesophageal varices, and to first disease decompensation during the follow-up period.

PATIENTS AND METHODS

A consecutive cohort of patients with compensated HCV cirrhosis and an available liver biopsy was prospectively enrolled at the Liver Unit of the University of Palermo. All patients gave written informed consent for the use of clinical data and for the histological evaluation.

Disease management

Liver biopsies were performed using Menghini needles of 16 gauge and were scored by a single pathologist using METAVIR score.[16] Only patients with METAVIR stage 4 were included in the study. On the same week of liver biopsy, all patients underwent disease assessment including the virological evaluation (quantitative HCV-RNA by reverse transcription-PCR using Cobas Amplicor HCV Monitor Test, v 2.0; Roche, Basel, Switzerland, and HCV genotyping by INNO-LiPA HCV II assay; Innogenetics, Zwijndrecht, Belgium), routine haematology (platelets and leucocytes count and haemoglobin levels), liver function tests (bilirubin, albumin, AST and ALT prothrombin time) and renal function tests (serum creatinine).

Upper GI endoscopy was performed within 3 months from histological diagnosis of liver cirrhosis and has been repeated every 2–3 years as indicated by guidelines.[17]

On serum stored at baseline genotyping for rs12979860 Single Nucleotide Polymorphism loked was carried out using the TaqMan SNP genotyping allelic discrimination method (Applied Biosystems, Foster City, CA, USA) as reported previously.[18]

Within not more than 3 months after liver biopsy, all patients received a course of PEG-IFN plus Ribavirin and the sustained virological response (SVR) was defined as undetectable HCV-RNA in serum 24 weeks after stopping therapy in accordance with international guidelines.[19]

During the period of observation, the abdominal US and a control of liver function tests (albumin, prothrombin time and bilirubin) were performed every 6 months.

Biopsy specimen study

For each paraffin-embedded liver biopsy sample, the total length (lengths of each fragment summed) was recorded and liver biopsies less than 15-mm long were excluded. The sections of each biopsy stained with picroSirius red were used for DIA which was performed by one author (V.C.). The equipment setup used consisted of a digital camera [Canon Powershot A640 (Canon, Middlesex, UK) attached to a close-up copystand with backlighting] connected to a compatible personal computer. Calibration of camera setup was: 154 × 154 pixels = 23 716 = 1 mm^2. After whole section digital image capture, CPA was measured with Zeiss Axiovision Rel 4.8 (Carl Zeiss, MicriImaging GmbH, Jena, Deutschland) image analysis software.

The CPA measurement included editing steps to eliminate image artefacts and structural collagen in large portal tracts (which do not represent disease-related liver fibrosis). Unfilled natural spaces such as vascular cavities and lymphoid aggregates were not included in the measurements. The software allows slider adjustment to view the output overlay at different detection thresholds for binary (grayscale); or colour (Red, Green, Blue). This method permits the optimization of the detection overlay by direct visual comparison with the original picroSirius red image.

We assessed intraobserver variability by repeating the CPA assessment using 10 biopsies (10% of cohort) randomly selected for a second evaluation by V.C. and interobserver error by using a different observer (D.C.) unaware of V.C.'s assessments, using a randomly selected group of 10 biopsies (10% of the cohort). Cohen's kappa coefficient has been reported.

Statistical analysis

All data were analyzed using the statistical package SPSS (version 15.0; SPSS, Chicago, IL, USA). Continuous variables were summarized as mean and standard deviation (s.d.), and categorical variables as frequency and percentage. Correlation between variables was evaluated by Spearman correlation. Significance testing was two sided and set to <0.05.

Area under the receiver operating characteristic (AUROC) analysis was used to establish the best cut off value of CPA to predict the presence of oesophageal varices (OV) and LD.

Logistic regression analysis was used to determine independent associations with the presence of OV at baseline. Cox regression analysis was used to evaluate the variables associated with LD occurrence during the follow-up. LD was defined as ascites, variceal bleeding or hepatic encephalopathy. The baseline variables included in the analysis to determine the association with the presence of OV were age, gender, serum levels of AST and ALT, platelet count, albumin and bilirubin levels, prothrombin activity and CPA and the variables included in the Cox analysis to determine the association with the LD were age, gender, serum levels of AST and ALT, platelet count, albumin and bilirubin levels, prothrombin activity, CPA, OV and SVR to anti-viral therapy. The proportion of patients who experienced LD and the time of the event were evaluated by Kaplan–Meier curves and log-rank analysis.

A comparison between CPA value in patients who developed hepatocellular carcinoma (HCC) and who died during follow-up was performed.

RESULTS

One-hundred and twenty-seven HCV-RNA positive patients with histological stage 4 by METAVIR score were included. Seven biopsies were suboptimal (biopsy length shorter than 15 mm) and in two cases no more tissue was available to obtain a section for staining with Sirius red. One hundred and eighteen patients were analysed. Demographic, clinical and histological features of patients are shown in Table 1. Average mean of CPA in the assessable cohort was 17.1 ± 7.9%.

The mean CPA analyzed either in biopsies from 15 to 19 mm in length or those of 20 mm length or longer, were 17.5% and 16.7% ($P = 0.198$).

The concordance coefficients between intraobserver and interobserver evaluation were 0.96 and 0.95 respectively.

Table 1 | Demographic, clinical and histological features of 118 patients with compensated HCV cirrhosis prospectively evaluated by DIA

Variables	Results
Number of patients	118
Age (mean ± s.d.)	57.2 ± 9.1
Gender (% males)	68 (57.6)
AST-U/L (mean ± s.d.)	112.4 ± 64.9
ALT-U/L (mean ± s.d.)	152 ± 91.2
Platelets ($\times 10^9$/L) (mean ± s.d.)	143.1 ± 77.6
WBCs ($\times 10^3$/L) (mean ± s.d.)	6.18 ± 1749.0
Hb (g/dL) (mean ± s.d.)	14.6 ± 1.3
Prothrombin activity (%) (mean ± s.d.)	88.7 ± 18.0
Total Bilirubin (mg/dL) (mean ± s.d.)	0.9 ± 0.5
Albumin (g/dL) (mean ± s.d.)	4.1 ± 0.4
Spleen diameter (cm) (mean ± s.d.)	13.7 ± 3.1
Presence of oesophageal varices (%)	38 (32.2)
Presence of large oesophageal varices (%)	2 (1.7)
MHCV genotype (%)	
1	102 (86.4)
2–3	16 (13.6)
Genotypes of rs12979860 SNP	
C/C (%)	31 (26.3)
T/C or T/T (%)	87 (73.7)
CPA (%) (mean ± s.d.)	17.1 ± 7.9
SVR (%)	30 (25.6)

AST, aspartate aminotransferase; ALT, alanine aminotransferase; WBCs, white blood cells; Hb, haemoglobin; HCV, hepatitis C virus; CPA, collagen proportionate area; SVR, sustained virological response.

Quantitative variables are expressed as mean and s.d. and categorical variables as absolute number with percentage into brackets.

One hundred and two patients had a Child–Pugh score A 5 and 16 patients had a Child–Pugh score A 6. Thirty patients (30%) had OV at baseline. Mean CPA values were significantly lower in patients with Child–Pugh A 5 than Child–Pugh A 6 (16.5 ± 7.2% vs. 20.7 ± 12.4%, $P = 0.047$).

One hundred and two patients (86.4%), were infected by HCV genotype 1 and 16 (13.6%) by HCV genotype 2 or 3. Thirty patients (25.4%) obtained a SVR after a course of anti-viral therapy with Peg-IFN and Ribavirin. The mean CPA value in patients who obtained SVR was lower than in non-SVR patients (13.5 ± 5.7% vs. 18.3 ± 8.2%, $P = 0.004$).

Variables associated with the presence of OV

By univariate analysis, mean CPA was significantly different among patients with or without OV (14.8 ± 5.9% vs. 21.6 ± 9.5%, $P < 0.001$). By AUROC, the best CPA cut-off value for OV was 14% (AUROC 0.813, Sensitivity: 92%; Specificity: 75%; PPV: 64%; NPV 95%) (Figure 1a). The rate of patients with CPA ≥14% was significantly higher in the group of patients with OV (94.7%) than in those without OV (37.5%), $P < 0.001$. Platelet counts were marginally related to OV (128.6×10^9/L ± 43.2×10^9/L vs. 149.7×10^9/L ± 88.4×10^9/L; $P = 0.091$). By multivariate logistic analysis CPA ≥14% was the unique variable associated with OV (OR: 28.32, 95% CI: 6.30–127.28; $P < 0.001$) (Table 2).

We also verify the role of CPA to predict the presence of OV in the 88 patients who did not achieve SVR. By univariate and multivariate analysis CPA ≥14% remained the unique variable independently related to presence of OV (OR: 34.58, 95% CI: 4.41–271.42; $P = 0.001$) (Table 3).

Analysing the 88 patients who did not have OV at baseline we observed that mean CPA of patients who carried on to not have OV during the follow-up was lower with respect the 31 patients who had developed OV (13.6 ± 5.6% vs. 15.7 ± 6.6%). However this difference did not achieve the statistical significance ($P = 0.121$).

Variables associated with the risk of LD

The median follow-up was 72 months (IQR 47–91). Seventeen patients developed decompensation (12 ascites, 3 EPS and 2 OV bleeding).

By univariate analysis, mean CPA was significantly different among patients with or without development of LD (23.5 ± 8.0% vs. 15.9 ± 7.5%; $P < 0.001$) (Table 3). Also platelet count (107.3×10^9/L ± 40.0×10^9/L vs. 142.7×10^9/L ± 44.4×10^9/L; $P = 0.003$), albumin (3.7 ± 0.5 vs. 4.2 ± 0.4 g/dL; $P < 0.001$), presence of OV (76.5% vs. 24.5%; $P < 0.001$) and absence of SVR after anti-viral therapy (94.1% vs. 71.4%; $P = 0.043$) were associated with an higher risk of decompensation. By AUROC analysis, the best CPA cut-off value for decompensation during follow-up was 18.0% (AUROC: 0.815, Sensitivity: 84%; Specificity: 78%; PPV: 40%; NPV 97%) (Figure 1b). By Kaplan–Meyer analysis, we found that patients with a CPA less than 18.0% had a longer decompensation-free survival (119.2 months, 95% CI: 113.8–124.8 months) as compared to those with CPA equal or above 18.0% (90.8 months, 95% CI: 74.9–106.7 months), Log rank test: $P < 0.001$ (Figure 2). By Cox regression multivariate analysis, CPA ≥18% (HR: 3.99, 95% CI: 1.04–11.45; $P = 0.036$), albumin (HR: 0.12, 95% CI: 0.04–0.43; $P = 0.001$) and presence of OV (HR: 8.15, 95% CI: 2.31–28.78; $P = 0.001$) were independently associated with LD (Table 4).

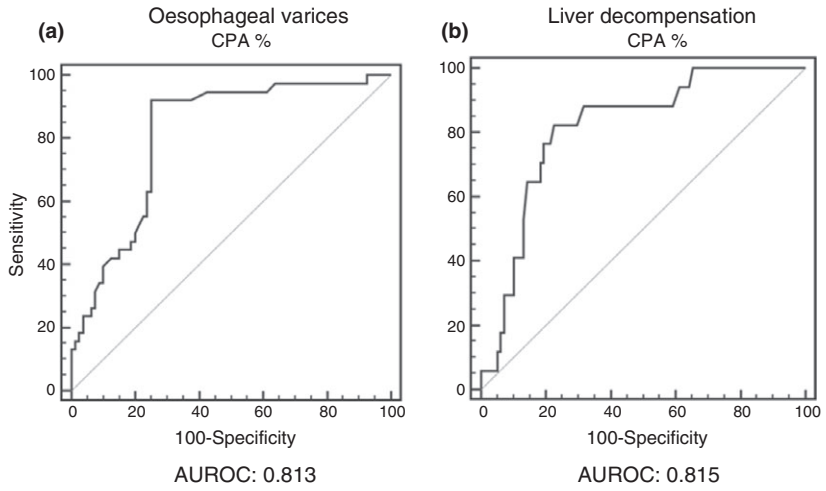

Figure 1 | AUROCs of CPA for diagnosis of OV and Liver decompensation in patients with HCV-related cirrhosis evaluated by DIA. (a) Receiver operating characteristic (ROC) curve showing the prediction of oesophageal varices with CPA in patients with HCV-related cirrhosis. The area under the ROC curve is 0.813. (b) Receiver operating characteristic (ROC) curve showing the prediction of liver decompensation with CPA in patients with HCV-related cirrhosis. The area under the ROC curve is 0.815.

Table 2 | Variables associated with oesophageal varices (OV) of 118 patients with histological diagnosis of HCV cirrhosis prospectively evaluated by CPA

Variables	No OV 80 pts (67.8%)	O 38 pts (32.2%)	Univariate analysis *P*-value	Multivariate analysis OR (95% CI)	*P*-value
Age (mean ± s.d.)	56.8 ± 9.0	58.1 ± 9.4	0.460	–	
Gender (% males)	45 (56.2)	23 (60.5)	0.660	–	
Platelets ($\times 10^9$/L, mean ± s.d.)	149.7 ± 88.4	128.6 ± 43.2	0.091	0.99 (0.98–1.01)	0.563
AST (IU/L, mean ± s.d.)	112.8 ± 65.8	112.0 ± 64.1	0.950	–	
ALT (IU/L, mean ± s.d.)	159.6 ± 96.2	136.4 ± 83.2	0.225	–	
Prothrombin time (%, mean ± s.d.)	90.2 ± 14.4	91.7 ± 9.3	0.911	–	
Bilirubin (mg/dL, mean ± s.d.)	0.9 ± 0.5	0.9 ± 0.5	0.522	–	
Albumin (g/dL, mean ± s.d.)	4.1 ± 0.5	4.0 ± 0.4	0.122	–	
CPA ≥14%	30 (37.5)	36 (94.7)	<0.001	28.32 (6.30–127.28)	**<0.001**

Quantitative variables are expressed as mean and standard deviation and categorical variables as absolute number with percentage into brackets.

By univariate analysis CPA value is significantly associated to presence of OV. The analysis shows a marginal association with platelets ($P < 0.1$). By multivariate analysis CPA equal or higher 14% is the unique variables associated with presence of OV.

AST, aspartate aminotransferase; ALT, alanine aminotransferase; CPA, collagen proportionate area; OV, oesophageal varices; OR, odds ratio; CI, confidence interval.

Bold data identify the statistical significant p values.

The same analysis was also performed among the 88 patients who did not achieve SVR after antiviral therapy. Similarly to the whole cohort, at the univariate analysis the variables related to LD were platelets, albumin value, presence of oesophageal varices and CPA ≥18%. By multivariate Cox regression analysis, albumin, presence of OV and CPA ≥18% remained the independent predictors of LD (Table 5).

Area under the receiver operating characteristic analysis of presence of oesophageal varices and albumin value for LD are shown in Figure S1. For both variables, the area under the curve is lower than the AUROC of CPA.

Table 3 | Variables associated with oesophageal varices (OV) of 88 patients with histological diagnosis of HCV cirrhosis prospectively evaluated by CPA and non-responder to anti-viral therapy with Peg-interferon and Ribavirin

Variables	No OV 56 pts (63.6%)	OV 32 pts (36.4%)	Univariate analysis *P*-value	Multivariate analysis OR (95% CI)	*P*-value
Age (mean ± s.d.)	56.6 ± 9.2	58.5 ± 9.0	0.362	–	
Gender (% males)	31 (55.4)	18 (56.3)	0.992	–	
Platelets ($\times 10^9$/L, mean ± s.d.)	135.0 ± 38.7	120.9 ± 38.3	0.104	–	
AST (IU/L, mean ± s.d.)	107.6 ± 57.1	114.0 ± 67.5	0.652	–	
ALT (IU/L, mean ± s.d.)	145.2 ± 81.7	139.2 ± 82.4	0.761	–	
Prothrombin time (%, mean ± s.d.)	89.0 ± 14.0	92.4 ± 9.5	0.220	–	
Bilirubin (mg/dL, mean ± s.d.)	0.9 ± 0.4	0.9 ± 0.4	0.605	–	
Albumin (g/dL, mean ± s.d.)	4.1 ± 0.4	4.0 ± 0.4	0.166	–	
CPA ≥ 14%	26 (46.4)	31 (96.9)	<0.001	34.58 (4.41–271.42)	**0.001**

Quantitative variables are expressed as mean and standard deviation and categorical variables as absolute number with percentage into brackets.

Both univariate and multivariate analysis show that CPA equal or higher 14% is the unique variables associated with presence of OV.

AST, aspartate aminotransferase; ALT, alanine aminotransferase; CPA, collagen proportionate area; OV, oesophageal varices; OR, odds ratio; CI, confidence interval.

Bold in all tables identify the statistical significant p values.

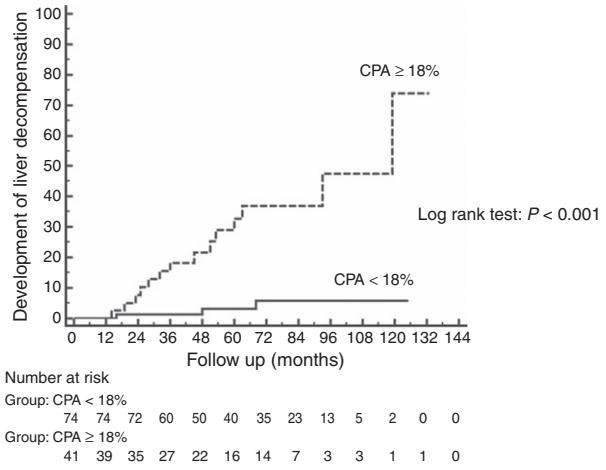

Figure 2 | Incidence of Liver Decompensation in patients with HCV cirrhosis according to CPA value. Cumulative incidence of first episode of liver decompensation (LD) in patients with CPA lower than 18% (continuous line) and in patients with CPA ≥18% (dashed line), By Kaplan–Meyer analysis, the patients with a CPA less than 18.0% had a longer decompensation-free survival (119.2 months, 95% CI: 113.8–124.8 months) as compared to those with CPA equal or above 18.0% (90.8 months, 95% CI: 74.9–106.7 months), log rank test: $P < 0.001$.

Collagen proportionate area was not associated with the risk of developing HCC since the mean CPA value in the eight patients with HCC during follow-up was comparable to those not developing HCC (18.0 ± 10.8% vs. 17.0 ± 7.8%, $P = 0.729$).

During the follow-up 12 patients (10.2%) died. Therefore, we have performed a Kaplan–Meyer analysis to predict mortality in this cohort of patients and we have found that patients with a CPA less than 18.0% had a longer survival (125.5 months, 95% CI: 119.3–131.7 months) as compared to those with CPA equal or above 18.0% (108.3 months, 95% CI: 94.6–122.0 months). This difference is significant by Log rank test: $P = 0.025$.

DISCUSSION

Quantitative assessment of fibrosis on liver biopsies from cirrhotic livers by measurement of the CPA allows to split the prognosis of compensated cirrhosis in different subclasses. Its correlation with HVPG has been shown in the setting of post-transplanted HCV patients[12] but until now CPA had not been evaluated in the setting of compensated HCV cirrhosis with the aim to identify its correlation with endoscopic signs of portal hypertension, and occurrence of LD.

Table 4 | Risk factors for liver decompensation (LD) by Cox multivariate model in 118 patients with biopsy proven HCV cirrhosis, prospectively evaluated by CPA

	No LD 98 pts* (85.6%)	LD 17 pts (14.4%)	Univariate analysis *P*-value	Multivariate analysis HR (95% CI)	*P*-value
Age (years, mean ± s.d.)	56.5 ± 9.4	60.0 ± 7.2	0.148	–	
Gender (% males)	58 (59.1)	7 (41.1)	0.153	–	
Platelets (×109/L, mean ± s.d.)	142.7 ± 44.4	107.3 ± 40.0	0.003	1.00 (0.98–1.02)	0.223
AST (IU/L, mean ± s.d.)	113.2 ± 67.1	108.8 ± 53.2	0.800		
ALT (IU/L, mean ± s.d.)	155.2 ± 94.1	133.6 ± 72.2	0.389		
Prothrombin time (%, mean ± s.d.)	90.2 ± 13.3	93.7 ± 10.4	0.325		
Bilirubin (mg/dL, mean ± s.d.)	0.9 ± 0.4	1.0 ± 0.5	0.258		
Albumin (g/dL, mean ± s.d.)	4.2 ± 0.4	3.7 ± 0.5	<0.001	0.12 (0.04–0.43)	**0.001**
CPA ≥18% (%)	28 (28.6)	14 (82.3)	<0.001	3.99 (1.04–11.45)	**0.036**
Presence of oesophageal varices (%)	24 (24.5)	13 (76.5)	<0.001	8.15 (2.31–28.78)	**0.001**
No SVR (%)	70 (71.4)	16 (94.1)	0.043	3.68 (0.40–38.05)	0.244

Quantitative variables are expressed as mean and standard deviation and categorical variables as absolute number with percentage into brackets.

By univariate Cox analysis platelet count, albumin value, CPA ≥18%, presence of OV and absence of virological response are associated with development of LD (all $P < 0.05$). The independent predictors of LD in the whole cohort of patients are albumin value, CPA ≥18% and presence of OV (all $P < 0.05$ by multivariate Cox regression analysis).

AST, aspartate aminotransferase; ALT, alanine aminotransferase; CPA, collagen proportionate area; SVR, sustained virological response; LD, liver decompensation; OR, odds ratio; CI, confidence interval.

* Three patients dropped out during follow-up.

Bold in all tables identify the statistical significant p values.

Our study, performed in a referral centre for the treatment and management of patients with chronic liver disease, have evaluated the CPA in a homogeneous cohort of patients with histological diagnosis of HCV compensated cirrhosis. Thirty-two percent of patients had oesophageal varices and 14.4% developed LD. Our results show that there is a higher CPA value in patients with Child–Pugh A6 than A5 demonstrating a better discriminative ability of CPA to differentiate between these two close classes of prognosis. Furthermore, a significant CPA difference was shown between patients with and without OV and the multivariate logistic analysis showed that CPA was the sole variable independently associated to the presence of OV. The CPA cut-off of 14% had a high sensitivity and negative predictive value to diagnose OV. This evidence confirms the direct correlation between CPA and portal hypertension suggesting its ability to identify different classes within the stage of compensated cirrhosis.

Moreover, liver CPA in the baseline biopsy was correlated with the occurrence of decompensation during a median follow-up of 72 months, and in particular the best cut off value of 18% of CPA was related to a higher risk of LD together with albumin values and presence of OV. The strength of this association was confirmed by the absence of SVR between the variables independently associated with LD, suggesting that patients with less fibrosis have a lower risk to develop decompensation in the short term regardless of the virological outcome of treatment. Thus, our data suggest that CPA is better than semiquantitative histological scores which are not able to describe beyond cirrhosis. CPA will be useful to stratify the risk of decompensation in cirrhosis, and help to chose and allocate patients correctly for anti-viral therapy. This may become particularly relevant at a time when 'informed deferral' of therapy is increasingly being considered in these patients in the wake of high expectations for IFN free regimens.[20] In the past years, many studies investigating risk factors for disease progression in patients with CHC have been performed and Konerman *et al.* have systematically summarised the results in a single document.[21]

The conclusion of this review demonstrated that even if a lot of studies have identified the factors associated with histological and/or clinical progression in CHC (platelets count, bilirubin, albumin, liver steatosis, etc.), we need to analyze cohorts of more characterised patients and follow them for a sufficiently long duration to can develop prediction models. Furthermore, no studies using CPA as predictor of disease progression have

Table 5 | Risk factors for liver decompensation (LD) by Cox multivariate model in 88 patients with biopsy proven HCV cirrhosis, prospectively evaluated by CPA and without response to anti-viral therapy with Peg-interferon and Ribavirin

	No LD 71 pts* (81.8%)	LD 16 pts (18.2%)	Univariate analysis *P*-value	Multivariate analysis HR (95% CI)	*P*-value
Age (years, mean ± s.d.)	56.6 ± 9.5	60.1 ± 7.2	0.173	–	
Gender (% males)	42 (59.2)	7 (43.8)	0.160	–	
Platelets ($\times 10^9$/L, mean ± s.d.)	135.3 ± 37.2	107.1 ± 38.8	0.003	0.99 (0.97–1.01)	0.246
AST (IU/L, mean ± s.d.)	110.4 ± 63.0	109.0 ± 52.9	0.928		
ALT (IU/L, mean ± s.d.)	145.2 ± 84.0	133.9 ± 72.2	0.617		
Prothrombin time (%, mean ± s.d.)	89.4 ± 13.0	93.1 ± 10.4	0.236		
Bilirubin (mg/dL, mean ± s.d.)	0.8 ± 0.3	1.0 ± 0.4	0.261		
Albumin (g/dL, mean ± s.d.)	4.1 ± 0.4	3.7 ± 0.5	<0.001	0.06 (0.01–0.27)	**<0.001**
CPA ≥18% (%)	23 (32.4)	14 (87.5)	<0.001	4.82 (1.28–18.16)	**0.020**
Presence of oesophageal varices (%)	19 (26.8)	13 (81.3)	0.007	13.9 (3.15–61.29)	**0.001**

Quantitative variables are expressed as mean and standard deviation and categorical variables as absolute number with percentage into brackets.

In patients who did not achieve SVR, platelet count, albumin value, CPA ≥18%, and presence of OV are associated with LD ($P < 0.05$ by univariate Cox analysis). The factors independently associated with LD by multivariate Cox regression analysis, are albumin value, CPA ≥18% and presence of OV (all $P < 0.05$).

AST, aspartate aminotransferase; ALT, alanine aminotransferase; CPA, collagen proportionate area; LD, liver decompensation; OR, odds ratio; CI, confidence interval.

* One no SVR patient dropped out during follow-up.

Bold in all tables identify the statistical significant p values.

been analyzed, therefore our study which enrolled an homogenous cohort of patient with a single aetiology of compensated cirrhosis, followed up for long time may be helpful to evaluate the prognostic role of this new predictor of clinical progression. Similarly, till now a systematic assessment of the available data about the role of non-invasive imaging modalities and risk of decompensation has not been performed. Robic *et al.*[22] have demonstrated that liver stiffness by TE is effective as HVPG in predicting clinical decompensation in patients with chronic liver disease. Moreover, in the last years, also liver stiffness measured by magnetic resonance elastography has been associated both with advanced fibrosis[23] and LD[24] in patients with chronic liver disease proving the ability of these techniques in sub-classify liver cirrhosis similarly to CPA. Furthermore, the first two studies.[10, 11] which have correlated liver stiffness value with CPA have found a significant correlation between the two parameters suggesting that a quantitative measurement of liver fibrosis as CPA could represent a better reference for non-invasive methods of liver fibrosis assessment. The risk of developing HCC was not associated with CPA. This result was not unexpected. Indeed, in a larger cohort of clinical or histological proven HCV patients with cirrhosis enrolled in our centre, we had already observed that the variables related to portal hypertension and liver function (presence of OV, platelet count, albumin value) did not influence the rate of development of HCC,[25] which seems to be related to other variables including age, gender and liver necroinflammation. Moreover, we did not find a significant difference in CPA values between patients who developed *de novo* varices, during follow-up or not. A possible explanation of this result could be the high percentage of very early stage of cirrhosis in patients who underwent liver biopsy. Indeed, in comparison with data from literature,[2, 26] we observed a lower rate of decompensation in the whole cohort (2.4% per year) and in the group of patients without OV (0.8% per year). This is most likely due to a selection bias, as all patients underwent liver biopsy to diagnose cirrhosis, especially because clear clinical signs of liver cirrhosis were not evident at baseline. Also the absence of significant correlation between platelet count and OV at multivariate analysis suggests a selection of a cohort with very early cirrhosis which is one limitation of this study.

A larger cohort of patient with cirrhosis and clinical evidence of portal hypertension (possibly using biopsies

taken by a transjugular route) would represent an interesting setting to further elaborate on our data and to further explore the prognostic role of CPA.

In conclusion, our study has confirmed that CPA measurement by DIA is a simple technique which can be used together with semiquantitative histological evaluation to better assess liver fibrosis and prognosis of patients with cirrhosis as well as chronic liver disease.

AUTHORSHIP

Guarantor of the article: Vincenza Calvaruso.

Author contributions: V. Calvaruso: designed the study, measured CPA, and contributed to data acquisition, is responsible for writing the manuscript and responsible for statistical analysis; V. Di Marco: participated in patient management and in writing the manuscript; M.G. Bavetta, E. Conte, F. Bronte and F. Simone: participated in patient management and data collection; D. Cabibi: is responsible for histological evaluation of liver specimens; A.K. Burrroughs: designed the study and participating in writing the manuscript; A. Craxì: designed the study and participated in writing the manuscript and have seen and approved the final version.

All authors have seen and approved the final version of the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. CPA values according to METAVIR stage of fibrosis in 97 patients with histological diagnosis of chronic hepatitis C.

Figure S1. AUROCs of OV and albumin for diagnosis of liver decompensation. Receiver operating characteristic (ROC) curves showing the performance of oesophageal varices and albumin in predicting of liver decompensation in patients with HCV related cirrhosis. The area under the ROC curves are 0.737 and 0.759 respectively.

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